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Serial No.: 09/439,594

Filed: November 12, 1999

Page 3 [Amendment Under 37 CFR § 1.115 (In Response To The
December 20, 2000 Office Action – March 20, 2002)]



AMEND THE ABOVE-IDENTIFIED APPLICATION AS FOLLOWS:

In The Title:

Change the title of the invention to:

-- DETECTING THE PRESENCE OF SPECIFIC TARGET NUCLEIC
ACID SEQUENCES THROUGH STEM-LOOP FORMATION --

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In The Claims:

Add new claims 146-200 as follows:

146. (NEW) A process for detecting the presence of a specific target nucleic acid sequence comprising the steps of:

1) providing one or more first initial primers or first nucleic acid constructs comprising two segments:

(A) a first segment (i) being substantially complementary to a portion of said specific target nucleic acid sequence and (ii) capable of template dependent extension: and

(B) a second segment being (i) substantially non-identical to said first segment, (ii) substantially identical to a portion of said specific target nucleic acid sequence, (iii) substantially complementary to sequences that are synthesized by extension of the first segment of said first initial primers or first nucleic acid constructs with said specific target nucleic acid sequences as a template and where in the absence of a denaturation

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step, the first initial primers or first nucleic acid constructs being capable of participation in the formation of a stem-loop structure after said specific target nucleic acid sequence is used as a template for extension;

2) mixing said first initial primer or nucleic acid constructs with substrates, buffer, a template-dependent polymerizing enzyme and a sample to be tested for the presence of said specific target nucleic acid;

3) incubating said mixture under temperature conditions sufficient for binding of said first initial primers or first nucleic acid constructs to said specific target nucleic acid sequence, extension of said first initial primers or first nucleic acid constructs and formation of stem-loop structures;

4) forming at least one stem-loop structure by

(a) self-annealing between said second segment of said first initial primer or first nucleic acid construct and a segment derived from target template dependent extension of the first segment of said first initial primer or first nucleic acid construct, and

(b) separating said first segment of said first initial primer or first nucleic acid construct from said specific target nucleic acid sequence, and

5) detecting the presence of said stem-loop structures formed in said forming step (4), thereby detecting the presence of said specific target nucleic acid sequence or its complement.

147. (NEW) The process of claim 146, wherein prior to or after said mixing step 2) any double-stranded nucleic acids in said sample have been rendered single-stranded.

148. (NEW) The process of claim 147, wherein said single-stranded rendering has been carried out by heat denaturation.

149. (NEW) The process of claim 146, wherein said template polymerizing enzyme comprises a member selected from the group consisting of DNA polymerase, RNA polymerase, reverse transcriptase, DNA ligase and a combination of any of the foregoing.

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150. (NEW) The process of claim 146, wherein said first initial primers, extended first initial primers, first nucleic acid constructs or extended first nucleic acid constructs comprise one or more moieties selected from the group consisting of modified nucleotides, non-nucleic acid polymers, branched nucleic acids, inverted nucleic acids, peptide nucleic acids, spacer groups, abasic sites, detectable labels and a combination of any of the foregoing.

151. (NEW) The process of claim 146, wherein said first initial primer or first nucleic acid construct or said extended first initial primer or first nucleic acid construct comprises one or more modified nucleotides having detectable labels.

152. (NEW) The process of claim 150 or 151, wherein said detectable labels are selected from the group consisting of haptens, ligands, receptors, fluorescein, rhodamine, coumarin, fluoresceinated molecules, infra-rad fluorescent groups, chemiluminescent moieties, energy transfer systems, enzymes and a combination of any of the foregoing.

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153. (NEW) The process of any of claims 146, 147, 148, 149, 150 or 151, wherein said mixing step 2), the sample is the product of an amplification reaction.

154. (NEW) The process of any of claims 146, 147, 148, 149, 150 or 151, wherein said mixing step 2), the sample has not previously undergone amplification or has been subjected to an amplification process.

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155. (NEW) The process of claim 152, wherein said mixing step 2), the sample is the product of an amplification reaction.

156. (NEW) The process of claim 152, wherein said mixing step 2), the sample has not previously undergone amplification or has been subjected to an amplification process.

157. (NEW) The process of claim 146, wherein said providing step 1) at least one second initial primer or second nucleic acid construct is provided, wherein said second initial primer or second nucleic acid construct comprises a first segment substantially complementary to sequences that are synthesized after extension of said first initial primer or first nucleic acid construct.

158. (NEW) The process of claim 157, wherein said second initial primer or second nucleic acid construct further comprises a second segment being (i) substantially non-identical to the first segment of said second initial primer or second nucleic acid construct; (ii) substantially identical to a portion of said extended first initial primer or first nucleic acid construct; and (iii) substantially complementary to sequences that are synthesized by extension of the first segment of said second initial primer or second nucleic acid construct with said extended first primer or nucleic acid construct as a template.

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159. (NEW) The process of claim 158, wherein said separating step 4)(b) is carried out by strand displacement, said strand displacement being carried out by extending at least one additional primer that binds to a site closer to the 3' end of said specific target nucleic acid sequence than the binding site for a first segment of a first initial primer or first nucleic acid construct or a second initial primer or second nucleic acid construct.

160. (NEW) The process of claim 157, wherein said second initial primers, extended initial primers, nucleic acid constructs or extended nucleic acid constructs comprise one or more moieties selected from the group consisting of modified nucleotides, non-nucleic acid polymers, branched nucleic acids, inverted nucleic acids, peptide nucleic acids, spacer groups, abasic sites, detectable labels and a combination of any of the foregoing.

161. (NEW) The process of claims 158 or 159, wherein said second initial primer or second nucleic acid construct or said extended second initial primer or extended second nucleic acid construct comprises one or more modified nucleotides having detectable labels.

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162. (NEW) The process of claims 160, wherein said detectable labels are selected from the group consisting of haptens, ligands, receptors, fluorescein, rhodamine, coumarin, fluoresceinated molecules, infra-rad fluorescent groups, chemiluminescent moieties, energy transfer systems, enzymes and a combination of any of the foregoing.

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163. (NEW) The process of claims 161, wherein said detectable labels are selected from the group consisting of haptens, ligands, receptors, fluorescein, rhodamine, coumarin, fluoresceinated molecules, infra-rad fluorescent groups, chemiluminescent moieties, energy transfer systems, enzymes and a combination of any of the foregoing.

164. (NEW) The process of claims 146, 157 or 158, wherein the detection of the formation of the stem-loop structure takes place after nucleic acid synthesis is substantially completed.

165. (NEW) The process of claims 146, 157 or 158, wherein the detection of the formation of the stem-loop structure takes place before nucleic acid synthesis is substantially completed.

166. (NEW) A process for detecting the presence of a specific target nucleic acid sequence comprising the steps of:

1) providing one or more first initial primers or first nucleic acid constructs comprising two segments:

(A) a first segment (i) being substantially complementary to a portion of said specific target nucleic acid sequence and (ii) capable of template dependent extension; and

(B) a second segment being (i) substantially non-identical to said first segment, (ii) substantially identical to a portion of said specific target nucleic acid sequence, (iii) substantially complementary to sequences that are synthesized by extension of the first segment of said first initial primers or first nucleic acid constructs with said specific target nucleic acid sequences as a template and wherein the first initial primers or first nucleic acid constructs being capable of participation in the formation of a stem-loop structure after said specific target nucleic acid sequence is used as a template for extension;

2) mixing said first initial primer or nucleic acid constructs with substrates, buffer, a template-dependent polymerizing enzyme and a sample to be tested for the presence of said specific target nucleic acid;

3) incubating said mixture under temperature conditions sufficient for binding of said first initial primers or first nucleic acid constructs to said specific target nucleic acid sequence, extension of said first initial primers or first nucleic acid constructs, and formation of stem-loop structures, and wherein said

extension is only carried out using first initial primers or nucleic acid constructs comprising said two segments;

- 4) forming at least one stem-loop structure by
- (a) self-annealing between said second segment of said first initial primer or first nucleic acid construct and a segment derived from target template dependent extension of the first segment of said first initial primer or first nucleic acid construct, and
 - (b) separating said first segment of said first initial primer or first nucleic acid construct from said specific target nucleic acid sequence,
- 5) detecting the presence of said stem-loop structures formed in said forming step (4), thereby detecting the presence of said specific target nucleic acid sequence or its complement.

167. (NEW) The process of claim 166, wherein prior to or after said mixing step 2) any double-stranded nucleic acids in said sample have been rendered single-stranded.

168. (NEW) The process of claim 166, wherein said single-stranded rendering has been carried out by heat denaturation.

169. (NEW) The process of claim 166, wherein said template polymerizing enzyme comprises a member selected from the group consisting of DNA polymerase, RNA polymerase, reverse transcriptase, DNA ligase and a combination of any of the foregoing.

170. (NEW) The process of claim 166, wherein said first initial primers, extended first initial primers, first nucleic acid constructs or extended first nucleic acid constructs comprise one or more moieties selected from the group consisting of modified nucleotides, non-nucleic acid polymers, branched nucleic acids, inverted nucleic acids, peptide nucleic acids, spacer groups, abasic sites, detectable labels and a combination of any of the foregoing.

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171. (NEW) The process of claim 166, wherein said first initial primer or first nucleic acid construct or said extended first initial primer or first nucleic acid construct comprises one or more modified nucleotides having detectable labels.

172. (NEW) The process of claim 170 or 171, wherein said detectable labels are selected from the group consisting of haptens, ligands, receptors, fluorescein, rhodamine, coumarin, fluoresceinated molecules, infra-rad fluorescent groups, chemiluminescent moieties, energy transfer systems, enzymes and a combination of any of the foregoing.

173. (NEW) The process of any of claims 166, 167, 168, 169, 170 or 171, wherein said mixing step 2), the sample is the product of an amplification reaction.

174. (NEW) The process of any of claim 172, wherein said mixing step 2), the sample has not previously undergone amplification or has been subjected to an amplification process.

175. (NEW) The process of claim 166, wherein said providing step 1) at least one second initial primer or second nucleic acid construct is provided, wherein said second initial primer or second nucleic acid construct comprises a first segment substantially complementary to sequences that are synthesized after extension of said first initial primer or first nucleic acid construct and is substantially non-identical to any sequences in said first initial primer or first nucleic acid construct.

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176. (NEW) The process of claim 175, wherein said second initial primer or second nucleic acid construct further comprises a second segment being (i) substantially non-identical to the first segment of said second initial primer or second nucleic acid construct; (ii) substantially identical to a portion of said extended first initial primer or first nucleic acid construct; and (iii) substantially complementary to sequences that are synthesized by extension of the first segment of said second initial primer or second nucleic acid construct with said extended first primer or nucleic acid construct as a template.

177. (NEW) The process of claim 166, wherein said separating step 4)(b) is carried out by strand displacement, said strand displacement being carried out by extending at least one additional primer that binds to a site closer to the 3' end of said specific target nucleic acid sequence than the binding site for a first segment of a first initial primer or first nucleic acid construct or a second initial primer or second nucleic acid construct.

178. (NEW) The process of claims 175, wherein said second initial primers, extended initial primers, nucleic acid constructs or extended nucleic acid constructs comprise one or more moieties selected from the group consisting of modified nucleotides, non-nucleic acid polymers, branched nucleic acids, inverted nucleic acids, peptide nucleic acids, spacer groups, abasic sites, detectable labels and a combination of any of the foregoing.

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Cod. 179. (NEW) The process of claims 175, wherein said second initial primer or second nucleic acid construct or said extended second initial primer or extended second nucleic acid construct comprises one or more modified nucleotides having detectable labels.

180. (NEW) The process of claims 179, wherein said detectable labels are selected from the group consisting of haptens, ligands, receptors, fluorescein, rhodamine, coumarin, fluoresceinated molecules, infra-rad fluorescent groups, chemiluminescent moieties, energy transfer systems, enzymes and a combination of any of the foregoing.

181. (NEW) The process of any of claims 166, 170, 171, 175, 176, 177, 178, 179 or 180, wherein the detection of the formation of the stem-loop structure takes place after nucleic acid synthesis is substantially completed.

182. (NEW) The process of any of claims 166, 170, 171, 175, 176, 177, 178, 179 or 180, wherein the detection of the formation of the stem-loop structure takes place before nucleic acid synthesis is substantially completed.

183. (NEW) A process for detecting the presence of a specific target nucleic acid sequence comprising the steps of:

1) providing one or more first initial primers or first nucleic acid constructs comprising two segments:

(A) a first segment (i) being substantially complementary to a portion of said specific target nucleic acid sequence and (ii) capable of template dependent extension; and

(B) a second segment being (i) substantially non-identical to said first segment, (ii) substantially identical to a portion of said specific target nucleic acid sequence, (iii) substantially complementary to sequences that are synthesized by extension of the first segment of said first initial primers or first nucleic acid constructs with said specific target nucleic acid sequences as a template to form a first extended nucleic acid strand, and wherein the first initial primers or first nucleic acid constructs being capable of participation in the formation of a stem-loop structure at one end of said first extended nucleic acid strand after said specific target nucleic acid sequence is used as a template for extension;

2) mixing said first initial primer or nucleic acid constructs with substrates, buffer, a template-dependent polymerizing enzyme and a sample to be tested for the presence of said specific target nucleic acid;

3) incubating said mixture under temperature conditions sufficient for binding of said first initial primers or first nucleic acid constructs to said specific

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target nucleic acid sequence, extension of said first initial primers or first nucleic acid constructs, and formation of stem-loop structures, and wherein said extension is only carried out using first initial primers or nucleic acid constructs comprising said two segments;

4) forming at least one stem-loop structure by

(a) self-annealing between said second segment of said first initial primer or first nucleic acid construct and a segment derived from target template dependent extension of the first segment of said first initial primer or first nucleic acid construct, and

(b) separating said first segment of said first initial primer or first nucleic acid construct from said specific target nucleic acid sequence,

5) detecting the presence of said stem-loop structures formed in said forming step (4), thereby detecting the presence of said specific target nucleic acid sequence or its complement.

184. (NEW) The process of claim 183, wherein prior to or after said mixing step 2) any double-stranded nucleic acids in said sample have been rendered single-stranded.

185. (NEW) The process of claim 183, wherein said single-stranded rendering has been carried out by heat denaturation.

186. (NEW) The process of claim 183, wherein said template polymerizing enzyme comprises a member selected from the group consisting of DNA polymerase, RNA polymerase, reverse transcriptase, DNA ligase and a combination of any of the foregoing.

187. (NEW) The process of claim 183, wherein said first initial primers, extended first initial primers, first nucleic acid constructs or extended first nucleic acid constructs comprise one or more moieties selected from the group consisting of modified nucleotides, non-nucleic acid polymers, branched nucleic acids, inverted nucleic acids, peptide nucleic acids, spacer groups, abasic sites, detectable labels and a combination of any of the foregoing.

188. (NEW) The process of claim 183, wherein said first initial primer or first nucleic acid construct or said extended first initial primer or first nucleic acid construct comprises one or more modified nucleotides having detectable labels.

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189. (NEW) The process of claim 187, wherein said detectable labels are selected from the group consisting of haptens, ligands, receptors, fluorescein, rhodamine, coumarin, fluoresceinated molecules, infra-rad fluorescent groups, chemiluminescent moieties, energy transfer systems, enzymes and a combination of any of the foregoing.

190. (NEW) The process of claim 188, wherein said detectable labels are selected from the group consisting of haptens, ligands, receptors, fluorescein, rhodamine, coumarin, fluoresceinated molecules, infra-rad fluorescent groups, chemiluminescent moieties, energy transfer systems, enzymes and a combination of any of the foregoing.

191. (NEW) The process of any of claims 183, 187, 188, 189 or 190, wherein said mixing step 2), the sample is the product of an amplification reaction.

192. (NEW) The process of any of claims 183, 187, 188, 189 or 190, wherein said mixing step 2), the sample has not previously undergone amplification or has been subjected to an amplification process.

193. (NEW) The process of claim 183, wherein said providing step 1) at least one second initial primer or second nucleic acid construct is provided, wherein said second initial primer or second nucleic acid construct comprises a first segment substantially complementary to sequences that are synthesized after extension of said first initial primer or first nucleic acid construct.

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COST. 194. (NEW) The process of claim 193, wherein said second initial primer or second nucleic acid construct further comprises a second segment being (i) substantially non-identical to the first segment of said second initial primer or second nucleic acid construct; (ii) substantially identical to a portion of said extended first initial primer or first nucleic acid construct; and (iii) substantially complementary to sequences that are synthesized by extension of the first segment of said second initial primer or second nucleic acid construct with said extended first primer or nucleic acid construct as a template.

195. (NEW) The process of claim 183, wherein said separating step 4)(b) is carried out by strand displacement, said strand displacement being carried out by extending at least one additional primer that binds to a site closer to the 3' end of said specific target nucleic acid sequence than the binding site for a first segment of a first initial primer or first nucleic acid construct or a second initial primer or second nucleic acid construct.

196. (NEW) The process of claim 193, wherein said second initial primers, extended initial primers, nucleic acid constructs or extended nucleic acid constructs comprise one or more moieties selected from the group consisting of modified nucleotides, non-nucleic acid polymers, branched nucleic acids, inverted nucleic acids, peptide nucleic acids, spacer groups, abasic sites, detectable labels and a combination of any of the foregoing.

197. (NEW) The process of claims 193, wherein said second initial primer or second nucleic acid construct or said extended second initial primer or extended second nucleic acid construct comprises one or more modified nucleotides having detectable labels.

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COD. 198. (NEW) The process of claims 193, wherein said detectable labels are selected from the group consisting of haptens, ligands, receptors, fluorescein, rhodamine, coumarin, fluoresceinated molecules, infra-rad fluorescent groups, chemiluminescent moieties, energy transfer systems, enzymes and a combination of any of the foregoing.

199. (NEW) The process of any of claims 183, 187, 188, 189, 190, 193, 194, 195, 196, 197 or 198, wherein the detection of the formation of the stem-loop structure takes place after nucleic acid synthesis is substantially completed.

200. (NEW) The process of any of claims 183, 187, 188, 189, 190, 193, 194, 195, 196, 197 or 198, wherein the detection of the formation of the stem-loop structure takes place before nucleic acid synthesis is substantially completed.
